Evolutionary-conserved behavioral plasticity enables context-dependent performance of mating behavior in *C. elegans*

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Abstract
Behavioral plasticity – the ability to adjust behavior based on the environment – helps animals to achieve their goals amid changing conditions. However, many innate behaviors, such as mating, are often considered to be stereotyped and genetically "programmed". Here we describe a remarkable case of behavioral plasticity in *C. elegans* – under a different set of naturalistic conditions the male uses a unique, previously undescribed set of behavioral steps for mating. Under standard lab conditions (agar plates with *E. coli*), the male performs parallel mating, when his tail is parallel with his partner's body. But when placed in a liquid medium the male performs spiral mating, a complex behavioral sequence, which involves wrapping his body around the partner. The performance of spiral mating does not require a long-term change in growing conditions but it improves with experience, which is indicative of learning. We show that this learning involves a critical period – a time window around the L4 - early adult stage, which coincides with the development of most male-specific neurons. By testing several wild isolates of *C. elegans* and other *Caenorhabditis* species we suggest that spiral mating is a plastic phenotype conserved across the genus, and which is "fixed" in some species via genetic accommodation.

Introduction

Behavior is flexible on different scales.

Here, we describe a remarkable case of behavioral plasticity in *C. elegans*. Under a different set of naturalistic growing conditions the male uses a unique, previously undescribed set of behavioral steps for mating. Under standard lab conditions (agar plates with *E. coli*), the male performs parallel mating, when his tail is parallel with his partner's body but when placed in a liquid medium the male performs spiral mating, a complex behavioral sequence, which involves wrapping his body around the partner. The performance of spiral mating does not require a long-term change in growing conditions. Adult males can switch between parallel and spiral mating when kept on plates or in liquid medium respectively. However, the performance of spiral mating improves with time spent in liquid medium in the presence of mating partners.

Results
We sought to investigate the performance of mating by male *C. elegans* under different sets of naturalistic conditions. Under standard lab conditions (agar plates with *E. coli*), the male performs parallel mating, when his tail is parallel with his partner's body (Figure 1A). Our initial observations showed that when placed in a liquid hydrogel-containing medium, the male does not perform parallel mating. Instead, upon contact with the hermaphrodite, the male wraps his body around her – a behavior resembling *spiral mating*, which is known from other nematode species (Figure 1B). Previously, obligate spiral mating has been observed only in four

Caenorhabditis species (Figure 1C). It has been assumed that spiral mating evolved twice in *Caenorhabditis*. Our results show that spiral mating is a plastic phenotype that can be induced in *C. elegans* by a simple environmental change.

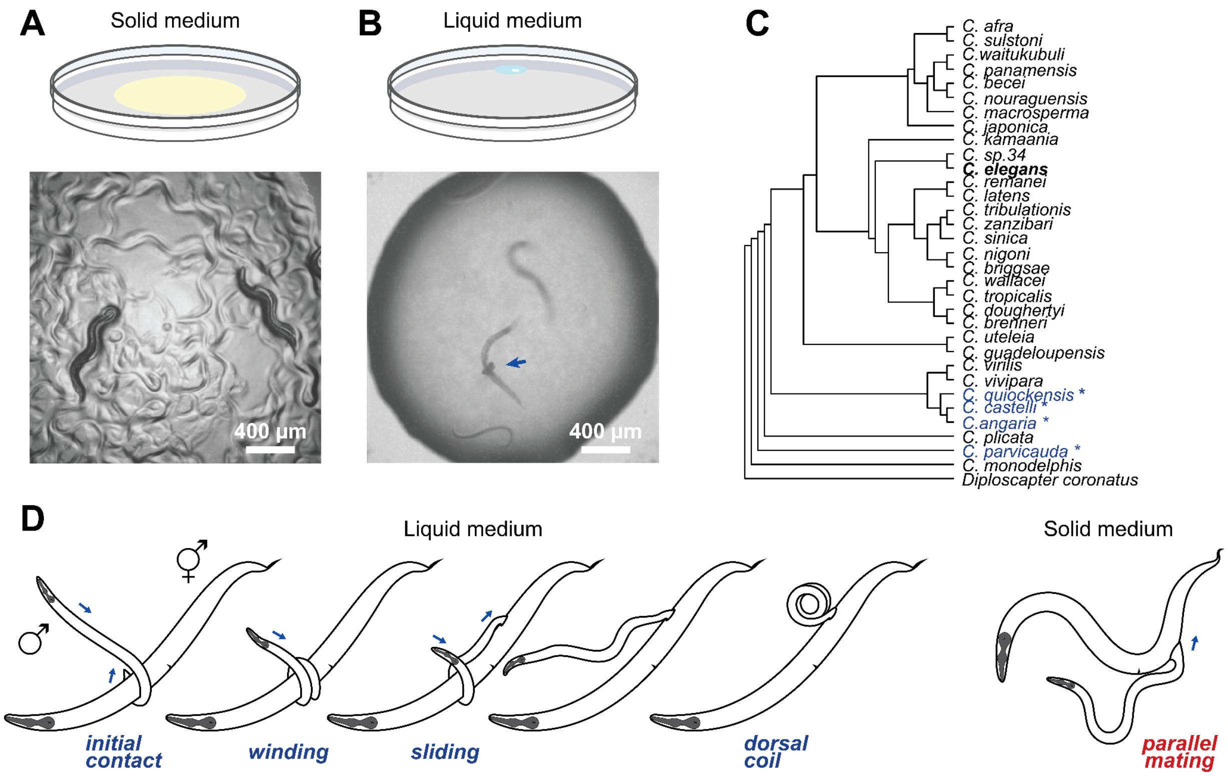

Figure 1. Behavioral plasticity in *C. elegans*. *C. elegans* male uses two different strategies for mating. On solid surfaces, the male performs parallel mating **(A)** whereas in liquid, the male uses spiral mating **(B)**. Blue arrow points at a male *C. elegans* wrapped around a hermaphrodite. **(C)** Phylogeny of *Caenorhabditis*; species with previously known spiral mating are indicated with asterisks, adapted from Stevens et al., 2019. **(D)** Spiral mating in liquid usually involves a stereotyped sequence of behavioral steps or *motifs*. Parallel mating is shown for comparison.

Video recordings of spiral mating of multiple *C. elegans* males revealed that this behavior involves a stereotyped sequence of behavioral steps or *motifs* (Figure 1D). First, the initial contact of the male tail with the hermaphrodite induces a sharp ventral flexion of the tail. When this first maneuver is successful, the male is able to grip the hermaphrodite with his tail. In the second step, the male winds himself around the hermaphrodite, forming a tight coil around her. In the third step, the male slides his tail along the hermaphrodite while keeping a grip on the hermaphrodite with his anterior end. In this motif, the winding grip of the anterior end enables the male to propel his tail backwards to a new location on the hermaphrodite while preventing the rest of the body sliding in the opposite direction. Sliding of the tail backwards coincides with the simultaneous unwinding of the anterior end, until it unwinds completely, at which point the male is only attached to the hermaphrodite by his tail. In the fourth step, after the sliding maneuver is completed, the male often bends his body dorsally, forming a coil. The last three steps of spiral mating can be repeated multiple times in a sequence, until the male either copulates or loses the hermaphrodite. The performance of spiral mating resembles concertina locomotion used by some arboreal snakes to climb tree branches. In the male *C. elegans*, spiral mating might help to mate in a liquid environment where the low substrate resistance presents a challenge for parallel mating. We conclude that spiral mating is a complex behavior consisting of separate behavioral motifs; it is distinct from parallel mating observed on solid surfaces, and likely represents an adaptation to low-friction environments.

To test whether males can mate successfully in liquid and produce progeny, we performed mating experiments. We used males and hermaphrodites that carried a mutation in *fog-2*, which prevented hermaphrodites from self-fertilizing. The males were divided into two groups. One group of males was raised on agar plates, whereas the other group was raised in liquid medium. In both cases hermaphrodites were present. We found that males can mate successfully in liquid medium, but only when they were cultured in liquid from the egg stage. Males grown on agar plates and transferred to liquid medium as adults failed to produce any progeny. These results show that (i) *C. elegans* males can mate successfully in liquid, and (ii) the ability to mate is dependent on prior experience.

Given that mating success depends on prior experience, we wanted to know if the performance specific steps of spiral mating differs between males grown in liquid and males transferred to liquid at the adult stage. Both males raised in liquid and on plates were able to perform all steps of spiral mating. However, males raised in liquid performed these steps more often – they showed more instances of prolonged contact with the hermaphrodite, winding around her, and sliding of the tail. We conclude that the performance of individual steps of spiral mating does not require a long-term change in growing conditions – adult males can switch from parallel to spiral mating when moved from plates to liquid medium respectively. However, the performance of spiral mating improves with time spent in liquid medium which is indicative of *learning*.

We next tested if the improved performance of spiral mating requires the presence of mating partners or if it can be attributed to a mere adaptation to the new (i.e. liquid) environment. We found that males cultured in liquid, with or without the presence of mating partners, showed more instances of motifs of spiral mating compared to males cultured on plates. However, males cultured in the presence of mating partners performed more motifs of spiral mating compared to males cultured alone. This suggests that the prior experience of mating in liquid (and not just an adaptation to the culture medium) results in the enhanced behavioral performance.

In many animals, learning involves critical periods during which learning is possible. To investigate whether experience-dependent improvement in mating performance involves a critical period, we transferred males between agar plates and liquid medium at different points in development. In particular, we focused on the time around the L4 larval stage when many neurons of the male-specific mating circuit are born…

We then tested whether this new case of behavioral plasticity is conserved across different wild isolates of *C. elegans*. We selected six genetically distinct isolates, and tested if they can perform spiral mating when placed in liquid medium. When cultured in liquid from the egg stage, all six isolates were able to perform spiral mating.

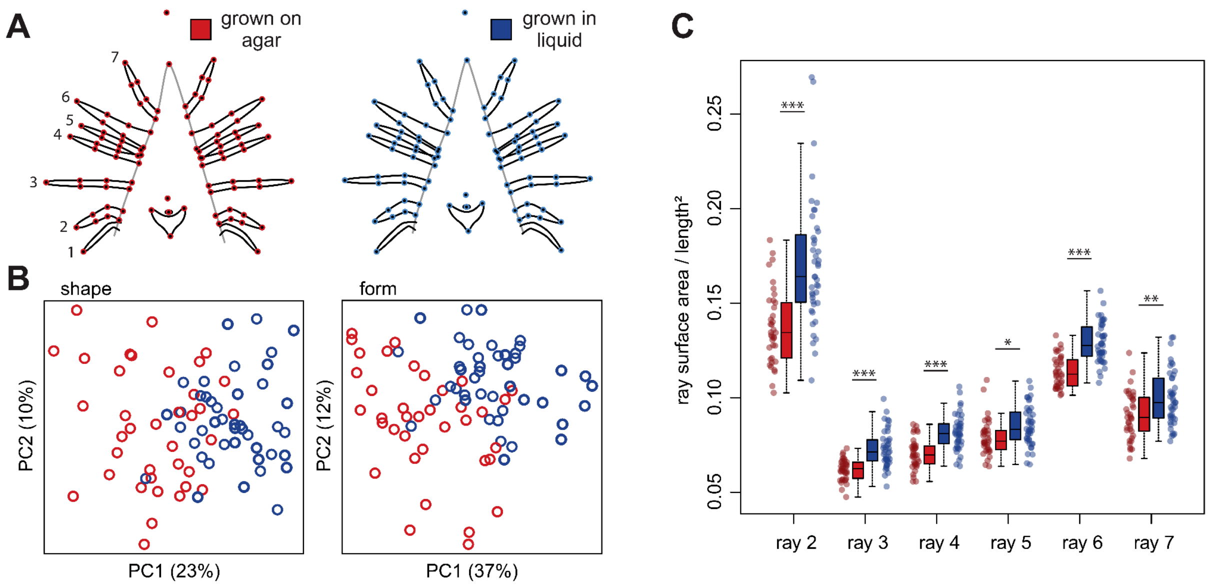

Figure 4. Different sensilla morphologies in different media. When cultured in liquid medium, males show a morphological change in the shape and form of their tail sensilla. **(A)** Average shapes of tail and sensilla structures in males grown on agar (left) and in liquid (right). Individual dots show morphological landmarks with lines outlining male rays and tail structures. **(B)** Males grown on agar and in liquid medium tend to occupy different subspaces of the Procrustes morphospace of shape (left) and form (shape+size, right). **(C)** Males grown in liquid (blue) have shorter and thicker sensory rays than males grown on agar (red). t-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Plastic behavioral phenotypes are often accompanied by a correlated morphological change. We used geometric morphometrics to compare morphologies of the male tail sensila between males grown on agar plates and in liquid medium (Figure 4A). Principal component analysis (PCA) of the tail shape and form showed that males raised using different media tend to occupy different regions of the Procrustes morphospace (Figure 4B). More detailed analysis showed that four ray pairs – 2, 3, 4, and 6 are especially affected (Figure 4C). Rays 2, 4, and 6 are shorter and thicker in males grown in liquid whereas ray 3 is also more curved in the middle. The functional significance of these morphological changes remains to be characterized, but the known roles of ray 2, 4, and 6 - innervating neurons – R2A, R4A, and R6A in the hermaphrodite recognition and scanning underscores their importance in mating.

Discussion

Acknowledgements

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Methods

Culture medium

Mating tests

Morphology

To test whether males cultured on agar and in liquid had morphological difference in their tail structures, we used geometric morphometrics, adapting an approach previously used for the nematode stomatal structures. Briefly,

Caenorhabditis strains

CB4088 *him-5* (*e1490*) V
CB4108 *fog-2* (*q71*) V

AB1 *C. elegans* wild isolate
CB4856 *C. elegans* wild isolate
JU1200 *C. elegans* wild isolate
MY16 *C. elegans* wild isolate
RC301 *C. elegans* wild isolate

References

Long-term imaging reveals behavioral plasticity during *C. elegans* dauer exit
Gripping during climbing of arboreal snakes may be safe but not economical
Soft Rod-Climbing Robot Inspired by Winding Locomotion of Snake
Life cycle and calculation of the intrinsic rate of natural increase
Life-History Traits of the Model Organism *Pristionchus pacificus* Recorded Using the Hanging Drop Method: Comparison with *Caenorhabditis elegans*
Comparative Studies on the Phylogeny and Systematics of the Rhabditidae (Nematoda)

Scraps

Species of *Caenorhabditis* nematodes have been thought to use either parallel or spiral modes of mating.
This motif enables the male to propel his tail backwards to a new location on the hermaphrodite while
being anchored to the hermaphrodite with the winding grip, preventing sliding in the opposite direction.
reminiscent of the way some arboreal snakes move in the tree canopy

Three-dimensional movements

Spiral mating is a plastic, environmentally induced phenotype

Spiral mating is a complex behavior consisting of stereotyped behavioral motifs

Spiral mating is conserved across wild isolates of *C. elegans*

Animals grown in liquid show improved performance of spiral mating

Spiral mating performance is experience-dependent

Growing in liquid medium induces morphological change in ray sensillae